BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

WEST Search History

DATE: Thursday, June 05, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,DWPI; PLUR=YES; OP=ADJ		
L9	l7 and l1	0	L9
L8	L7 and l3	4	L8
L7	OCH1 near3 (deficie$ or lack$ or knockout or muta$)	12	L7
L6	l4 and l3	0	L6
L5	L4 and l3	0	L5
L4	OCH near3 (deficie$ or lack$ or knockout)	8	L4
L3	mannosidase	1054	L3
L2	L1 and 30%	1	L2
L1	Man5GlcNAc2	4	L1

END OF SEARCH HISTORY

WEST Search History

DATE: Thursday, June 05, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,DWPI; PLUR=YES; OP=ADJ			
L8	L7 and l3	4	L8
L7	OCH1 near3 (deficie$ or lack$ or knockout or muta$)	12	L7
L6	l4 and l3	0	L6
L5	L4 and l3	0	L5
L4	OCH near3 (deficie$ or lack$ or knockout)	8	L4
L3	mannosidase	1054	L3
L2	L1 and 30%	1	L2
L1	Man5GlcNAc2	4	L1

END OF SEARCH HISTORY

$%^STN;HighlightOn= ***;HighlightOff=*** ;

Welcome to STN International! Enter x:x

LOGINID:ssspta1633cxq

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	Jun 03	New e-mail delivery for search results now available
NEWS 4	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 5	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 6	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 7	Sep 03	JAPIO has been reloaded and enhanced
NEWS 8	Sep 16	Experimental properties added to the REGISTRY file
NEWS 9	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS 10	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS 11	Oct 24	BEILSTEIN adds new search fields
NEWS 12	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 13	Nov 18	DKILIT has been renamed APOLLIT
NEWS 14	Nov 25	More calculated properties added to REGISTRY
NEWS 15	Dec 04	CSA files on STN
NEWS 16	Dec 17	PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 17	Dec 17	TOXCENTER enhanced with additional content
NEWS 18	Dec 17	Adis Clinical Trials Insight now available on STN
NEWS 19	Jan 29	Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC
NEWS 20	Feb 13	CANCERLIT is no longer being updated
NEWS 21	Feb 24	METADEX enhancements
NEWS 22	Feb 24	PCTGEN now available on STN
NEWS 23	Feb 24	TEMA now available on STN
NEWS 24	Feb 26	NTIS now allows simultaneous left and right truncation
NEWS 25	Feb 26	PCTFULL now contains images
NEWS 26	Mar 04	SDI PACKAGE for monthly delivery of multifile SDI results
NEWS 27	Mar 20	EVENTLINE will be removed from STN
NEWS 28	Mar 24	PATDPAFULL now available on STN
NEWS 29	Mar 24	Additional information for trade-named substances without structures available in REGISTRY
NEWS 30	Apr 11	Display formats in DGENE enhanced
NEWS 31	Apr 14	MEDLINE Reload
NEWS 32	Apr 17	Polymer searching in REGISTRY enhanced
NEWS 33	Apr 21	Indexing from 1947 to 1956 being added to records in CA/CAPLUS
NEWS 34	Apr 21	New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX
NEWS 35	Apr 28	RDISCLOSURE now available on STN
NEWS 36	May 05	Pharmacokinetic information and systematic chemical names added to PHAR
NEWS 37	May 15	MEDLINE file segment of TOXCENTER reloaded
NEWS 38	May 15	Supporter information for ENCOMPPAT and ENCOMPLIT updated

```
NEWS 39  May 16  CHEMREACT will be removed from STN
NEWS 40  May 19  Simultaneous left and right truncation added to WSCA
NEWS 41  May 19  RAPRA enhanced with new search field, simultaneous left and
                 right truncation

NEWS EXPRESS  April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT
              MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
              AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
NEWS HOURS    STN Operating Hours Plus Help Desk Availability
NEWS INTER    General Internet Information
NEWS LOGIN    Welcome Banner and News Items
NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:49:58 ON 05 JUN 2003

=> FIL BIOSIS EMBASE CAPLUS
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'BIOSIS' ENTERED AT 13:50:05 ON 05 JUN 2003
COPYRIGHT (C) 2003 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'EMBASE' ENTERED AT 13:50:05 ON 05 JUN 2003
COPYRIGHT (C) 2003 Elsevier Science B.V.  All rights reserved.

FILE 'CAPLUS' ENTERED AT 13:50:05 ON 05 JUN 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)

=> s Man5GlcNAc2
L1          438 MAN5GLCNAC2

=> s OCH1 (3a)(deficien? or lack? or knockout or muta?)
L2           31 OCH1 (3A)(DEFICIEN? OR LACK? OR KNOCKOUT OR MUTA?)

=> s MNN? (3a)(deficien? or lack? or knockout or muta?)
L3         1495 MNN? (3A)(DEFICIEN? OR LACK? OR KNOCKOUT OR MUTA?)

=> s l2 or l3
L4         1516 L2 OR L3

=> s l1 and l4
L5            6 L1 AND L4
```

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 3 DUP REM L5 (3 DUPLICATES REMOVED)

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS
AN 1998:672034 CAPLUS
DN 130:33642
TI Production of human compatible high mannose-type (***Man5GlcNAc2***) sugar chains in Saccharomyces cerevisiae
AU Chiba, Yasunori; Suzuki, Misa; Yoshida, Satoshi; Yoshida, Aruto; Ikenaga, Hiroshi; Takeuchi, Makoto; Jigami, Yoshifumi; Ichishima, Eiji
CS Central Laboratories for Key Technology, KIRIN Brewery Co., Ltd., Kanagawa, 236-0004, Japan
SO Journal of Biological Chemistry (1998), 273(41), 26298-26304
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB A yeast mutant capable of producing ***Man5GlcNAc2*** human compatible sugar chains on glycoproteins was constructed. An expression vector for .alpha.-1,2-mannosidase with the "HDEL" endoplasmic reticulum retention/retrieval tag was designed and expressed in Saccharomyces cerevisiae. An in vitro .alpha.-1,2-mannosidase assay and Western blot anal. showed that it was successfully localized in the endoplasmic reticulum. A triple mutant yeast lacking three glycosyltransferase activities was then transformed with an .alpha.-1,2-mannosidase expression vector. The oligosaccharide structures of carboxypeptidase Y as well as cell surface glycoproteins were analyzed, and the recombinant yeast was shown to produce a series of high mannose-type sugar chains including ***Man5GlcNAc2*** . This is the first report of a recombinant S. cerevisiae able to produce ***Man5GlcNAc2*** -oligosaccharides, the intermediate for hybrid-type and complex-type sugar chains.
RE.CNT 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 1997:366770 BIOSIS
DN PREV199799658703
TI MNN6, a member of the KRE2/MNT1 family, is the gene for mannosylphosphate transfer in Saccharomyces cerevisiae.
AU Wang, Xiao-Hui; Nakayama, Ken-Ichi; Shimma, Yoh-Ichi; Tanaka, Atsushi; Jigami, Yoshifumi (1)
CS (1) National Inst. Bioscience, Human Technol., 1-1 Higashi, Tsukuba, Ibaraki 305 Japan
SO Journal of Biological Chemistry, (1997) Vol. 272, No. 29, pp. 18117-18124.
ISSN: 0021-9258.
DT Article
LA English
AB In yeast Saccharomyces cerevisiae the N-linked sugar chain is modified at different positions by the addition of mannosylphosphate. The ***mnn6*** ***mutant*** is ***deficient*** in the mannosylphosphate transferase
activity toward mannotetraose (Karson, E. M., and Ballou, C. E. (1978) J.

Biol. Chem. 253,6484-6492). We have cloned the MNN6 gene by complementation. It has encoded a 446-amino acid polypeptide with the characteristics of type II membrane protein. The deduced Mnn6p showed a significant similarity to Kre2p/Mnt1p, a Golgi alpha-1,2-mannosyltransferase involved in O-glycosylation. The null ***mutant*** of ***MNN6*** showed a normal cell growth, less binding to Alcian blue, hypersensitivity to Calcoflour White and hygromycin B, and diminished mannosylphosphate transferase activity toward the endoplasmic reticulum core oligosaccharide acceptors (Man8GlcNAc2-PA and ***Man5GlcNAc2*** -PA) in vitro, suggesting the involvement of the MNN6 gene in the endoplasmic reticulum core oligosaccharide phosphorylation. However, no differences were observed in N-linked mannoprotein oligosaccharides between DELTA-och1 DELTA-mnn1 cells and DELTA-och1-DELTA-mnn1-DELTA-mnn6 cells, indicating the existence of redundant genes required for the core oligosaccharide phosphorylation. Based on a dramatic decrease in polymannose outer chain phosphorylation by MNN6 gene disruption and a determination of the mannosylphosphorylation site in the acceptor, it is postulated that the MNN6 gene may be a structural gene encoding a mannosylphosphate transferase, which recognizes any oligosaccharides with at least one alpha-1,2-linked mannobiose unit.

L6 ANSWER 3 OF 3 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 2
AN 93361722 EMBASE
DN 1993361722
TI Structure of the N-linked oligosaccharides that show the complete loss of .alpha.-1,6-polymannose outer chain from och1, och1 mnn1, and ***och1*** ***mnn1*** alg3 ***mutants*** of Saccharomyces cerevisiae.
AU Nakanishi-Shindo Y.; Nakayama K.-I.; Tanaka A.; Toda Y.; Jigami Y.
CS Natl. Bioscience/Human Tech. Inst., 1-1 Higashi,Tsukuba, Ibaraki 305, Japan
SO Journal of Biological Chemistry, (1993) 268/35 (26338-26345).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The periplasmic invertase was purified from Saccharomyces cerevisiae och1::LEU2 disruptant cells (.DELTA.och1), which have a defect in elongation of the outer chain attached to the N-linked core oligosaccharides (Nakayama, K., Nagasu, T., Shimma, Y., Kuromatsu, J., and Jigami, Y. (1992) EMBO J. 11, 2511-2519). Structural analysis of the pyridylaminated (PA) neutral oligosaccharides released by hydrazinolysis and N-acetylation confirmed that the ***och1*** ***mutation*** causes a complete loss of the .alpha.-1,6-polymannose outer chain, although the PA oligosaccharides (Man9GlcNAc2-PA and Man10GlcNAc2-PA), in which one or two .alpha.-1,3-linked mannose(s) attached to the endoplasmic reticulum (ER)-form core oligosaccharide (Man8GlcNAc2) were also detected. Analysis of the .DELTA.och1 mnn1 strain oligosaccharides released from total cell mannoprotein revealed that the .DELTA. ***och1*** ***mnn1*** ***mutant*** eliminates the .alpha.-1,3-mannose attached to the core and accumulates predominantly a single ER-form oligosaccharide species (Man8GlcNAc2), suggesting a potential use of this strain as a host cell to produce glycoproteins containing mammalian high mannose type oligosaccharides. The .DELTA. ***och1*** ***mnn1*** alg3 ***mutants*** accumulated ***Man5GlcNAc2*** and Man8GlcNAc2 in total

cell mannoprotein, confirming the lack of outer chain addition to the incomplete corelike oligosaccharide and the leaky phenotype of the alg3 mutation. All the results suggest that the OCH1 gene encodes an .alpha.-1,6-mannosyltransferase that is functional in the initiation of .alpha.-1,6-polymannose outer chain addition to the N-linked core oligosaccharide (***Man5GlcNAc2*** and Man8GlcNAc2) in yeast.

```
=> s 1, 2 mannosidase
L7          309 1, 2 MANNOSIDASE

=> d his

     (FILE 'HOME' ENTERED AT 13:49:58 ON 05 JUN 2003)

     FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 13:50:05 ON 05 JUN 2003
L1           438 S MAN5GLCNAC2
L2            31 S OCH1 (3A)(DEFICIEN? OR LACK? OR KNOCKOUT OR MUTA?)
L3          1495 S MNN? (3A)(DEFICIEN? OR LACK? OR KNOCKOUT OR MUTA?)
L4          1516 S L2 OR L3
L5             6 S L1 AND L4
L6             3 DUP REM L5 (3 DUPLICATES REMOVED)
L7           309 S 1, 2 MANNOSIDASE

=> s l1 and l7
L8           49 L1 AND L7

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9            24 DUP REM L8 (25 DUPLICATES REMOVED)

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 24 ANSWERS - CONTINUE? Y/(N):y

L9   ANSWER 1 OF 24  CAPLUS  COPYRIGHT 2003 ACS
AN   2003:179025  CAPLUS
TI   Modification of the N-glycosylation pathway of lower eukaryotes to a
     mammalian type
AU   Contreras, Roland H.; Vervecken, Wouter; Callewaert, Nico; Geysens,
     Steven; Kaigorodov, Vladimir
CS   Molecular Biology, Ghent University and VIB, B-9000 Gent, Belg.
SO   Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United
     States, March 23-27, 2003 (2003), BIOT-030 Publisher: American Chemical
     Society, Washington, D. C.
     CODEN: 69DSA4
DT   Conference; Meeting Abstract
LA   English
AB   Lower eukaryotes only synthesize N-glycans of the high-mannose type,
     whereas human glycoproteins have a very divers, complex type of N-glycans.
     Redirection of the fungal pathway, even to a simple hybrid or complex
     mammalian type requires several genetic interventions such as gene
     knock-outs and heterologous expression of mammalian glycosyl transferases.
     Furthermore, addnl. in vitro enzymic manipulations may be required.  In
     general, it seems that humanising N-glycans from filamentous fungi is an
     easier task than modifying yeast protein linked carbohydrates because the
     long alfa-1,6-arm apparently is absent.  The problem is reduced to
     importing an efficient alfa-  ***1***  ,  ***2***  -  ***mannosidase***
```

and addn. of complex sugar glycosyl transferases. We started a strategy to humanise the N-glycosylation pathway in the filamentous fungus Aspergillus niger NW195. In a first step a HDEL tagged a- ***1*** , ***2*** - ***mannosidase*** from Trichoderma reesei was introduced. The over expression of this enzyme lead to the conversion of the majority of the N-glycans to ***Man5GlcNAc2*** (ca. 80%). In a second step the over expression of human N&64979;acetylglucosaminyltransferase I lead to the detection of GlcNAcMan5GlcNAc2 structures. The percentage conversion of ***Man5GlcNAc2*** to GlcNAcMan5GlcNAc2 was inversely related to the amt. of protein that was synthesized, ranging from ca. 40% to merely all. Yeasts, like S. cerevisiae and P. pastoris, have an extra, long alfa-1,6-arm that is mannose and P-mannose rich. Eliminating this structure causes, at least in S. cerevisiae, a weak growth and temp. sensitive phenotype. Different procedures have been followed to knock out the OCH1 gene. Expression of alfa- ***1*** , ***2*** - ***mannosidase*** has also been obtained in combination with OCH1 knock out, resulting in very high yileds of ***Man5GlcNAc2*** N-linked structures. Furthermore, phosphodiester linked mannoses are, in general, unwanted in mammalian therapeutic situations. Knock out of phosphomannosyltransferases is required to eliminate these structures from yeast and fungal protein linked sugars. Cloning of the P. pastoris homologues of S. cerevisiae MNN4 and MNN6 genes is not an easy task. Ultimately, the terminal sialic acid should be added using in vitro procedures.

L9 ANSWER 2 OF 24 CAPLUS COPYRIGHT 2003 ACS
AN 2002:10683 CAPLUS
DN 136:80826
TI Methods for producing modified glycoproteins in lower eukaryotes expressing mammalian genes for enzymes of glycosylation
IN Gerngross, Tillman U.
PA Glycofi, Inc., USA
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002000879	A2	20020103	WO 2001-US20553	20010627
	WO 2002000879	A3	20020906		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 2002137134	A1	20020926	US 2001-892591	20010627
	EP 1297172	A2	20030402	EP 2001-954606	20010627
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI	US 2000-214358P	P	20000628		
	US 2000-215638P	P	20000630		
	US 2001-279997P	P	20010330		

WO 2001-US20553 W 20010627

AB Cell lines having genetically modified glycosylation pathways that allow them to carry out a sequence of enzymic reactions, which mimic the processing of glycoproteins in humans, have been developed. Recombinant proteins expressed in these engineered hosts yield glycoproteins more similar, if not substantially identical to their human counterparts. The lower eukaryotes, which ordinarily produce high-mannose contg. N-glycans, including unicellular and multicellular fungi are modified to produce N-glycans such as ***Man5GlcNAc2*** or other structures along human glycosylation pathways. This is achieved using a combination of engineering and/or selection of strains which: do not express certain enzymes, such as phospho mannosyltransferase, 1,6-mannosyltransferase, 1,3-mannosyltransferase and 1,2-mannosyltransferase, which create the undesirable complex structures characteristic of the fungal glycoproteins. The expressed exogenous enzymes selected either have optimal activity under the conditions present in the fungi where activity is desired, or which are targeted to an organelle where optimal activity is achieved. The said engineering and/or selection of strains combinations provide a method for genetically engineering eukaryote expressing multiple exogenous enzymes required to produce "human-like" glycoproteins.

L9 ANSWER 3 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1

AN 2002:214920 BIOSIS

DN PREV200200214920

TI Structure of Penicillium citrinum alpha1,2-mannosidase reveals the basis for differences in specificity of the endoplasmic reticulum and Golgi class I enzymes.

AU Lobsanov, Yuri D.; Vallee, Francois; Imberty, Anne; Yoshida, Takashi; Yip, Patrick; Herscovics, Annette; Howell, P. Lynne (1)

CS (1) Program in Structural Biology and Biochemistry, Research Institute, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, M5G 1X8: howell@sickkids.on.ca Canada

SO Journal of Biological Chemistry, (February 15, 2002) Vol. 277, No. 7, pp. 5620-5630. http://www.jbc.org/. print.
ISSN: 0021-9258.

DT Article

LA English

AB Class I alpha1,2-mannosidases (glycosylhydrolase family 47) are key enzymes in the maturation of N-glycans. This protein family includes two distinct enzymatically active subgroups. Subgroup 1 includes the yeast and human endoplasmic reticulum (ER) alpha1,2-mannosidases that primarily trim Man9GlcNAc2 to Man8GlcNAc2 isomer B whereas subgroup 2 includes mammalian Golgi alpha1,2-mannosidases IA, IB, and IC that trim Man9GlcNAc2 to ***Man5GlcNAc2*** via Man8GlcNAc2 isomers A and C. The structure of the catalytic domain of the subgroup 2 alpha1,2-mannosidase from Penicillium citrinum has been determined by molecular replacement at 2.2-ANG resolution. The fungal alpha1,2-mannosidase is an (alphaalpha)7-helix barrel, very similar to the subgroup 1 yeast (Vallee, F., Lipari, F., Yip, P., Sleno, B., Herscovics, A., and Howell, P. L. (2000) EMBO J. 19, 581-588) and human (Vallee, F., Karaveg, K., Herscovics, A., Moremen, K. W., and Howell, P. L. (2000) J. Biol. Chem. 275, 41287-41298) ER enzymes. The location of the conserved acidic residues of the catalytic site and the binding of the inhibitors, kifunensine and 1-deoxymannojirimycin, to the essential calcium ion are conserved in the fungal enzyme. However, there are major structural differences in the oligosaccharide binding site between the two alpha1,2-mannosidase subgroups. In the subgroup 1 enzymes,

an arginine residue plays a critical role in stabilizing the oligosaccharide substrate. In the fungal alpha1,2-mannosidase this arginine is replaced by glycine. This replacement and other sequence variations result in a more spacious carbohydrate binding site. Modeling studies of interactions between the yeast, human and fungal enzymes with different Man8GlcNAc2 isomers indicate that there is a greater degree of freedom to bind the oligosaccharide in the active site of the fungal enzyme than in the yeast and human ER alpha1,2-mannosidases.

L9 ANSWER 4 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:432114 BIOSIS
DN PREV200200432114
TI Tissue-specific expression of mammalian Golgi class I alpha1,2-mannosidases involved in N-glycan processing.
AU Tremblay, Linda Olive (1); Herscovics, Annette (1)
CS (1) McGill Cancer Centre, McGill University, 3655 Promenade Sir William Osler, Montreal, PQ, H3G 1Y6 Canada
SO FASEB Journal, (March 22, 2002) Vol. 16, No. 5, pp. A1178. http://www.fasebj.org/. print.
Meeting Info.: Annual Meeting of Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638.
DT Conference
LA English
AB Class I alpha1,2-mannosidases are a family of enzymes essential for the formation of mammalian complex and hybrid N-glycans. There are three Golgi alpha1,2-mannosidase orthologs (IA, IB and IC) that can form
Man5GlcNAc2 . Although they have similar specificities demonstrated
with recombinant enzyme, Northern blots show that these mannosidases have very different patterns of tissue-specific expression in adult mouse and human tissues and during mouse embryonic development. Expression of mouse Golgi alpha1,2-mannosidase IB is temporarily upregulated during postimplantation development whereas the expression of alpha1,2-mannosidase IA and IC is relatively constant. In order to further investigate its specific role alpha1,2-mannosidase IB gene targeting constructs were generated using the pflox vector (J. Clin. Invest. (1996) 97:1999-2002) and ES cells with an alpha1,2-mannosidase IB gene Type I and Type II recombination were isolated for the generation of systemic and conditional alpha1,2-mannosidase IB deletions, respectively.

L9 ANSWER 5 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:472646 BIOSIS
DN PREV200100472646
TI Structure and function of Class I alpha1,2-mannosidases involved in glycoprotein synthesis and endoplasmic reticulum quality control.
AU Herscovics, Annette (1)
CS (1) McGill Cancer Centre, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec, H3G 1Y6: annette@med.mcgill.ca Canada
SO Biochimie (Paris), (August, 2001) Vol. 83, No. 8, pp. 757-762. print.
ISSN: 0300-9084.
DT Article
LA English
SL English
AB Class I alpha1,2-mannosidases (glycosylhydrolase family 47) are conserved through eukaryotic evolution. This protein family comprises three

subgroups distinguished by their enzymatic properties. The first subgroup includes yeast (Saccharomyces cerevisiae) and human alpha1,2-mannosidases of the endoplasmic reticulum that primarily form Man8GlcNAc2 isomer B from Man9GlcNAc2. The second subgroup includes mammalian Golgi alpha1,2-mannosidases, as well as enzymes from insect cells and from filamentous fungi, that trim Man9GlcNAc2 to Man8GlcNAc2 isomers A and/or C intermediates toward the formation of ***Man5GlcNAc2*** . Yeast and mammalian proteins of the third subgroup have no enzyme activity with Man9GlncNA2 as substrate. The members of subgroups 1 and 3 participate in endoplasmic reticulum quality control and promote proteasomal degradation of misfolded glycoproteins. The yeast endoplasmic reticulum alpha1,2-mannosidase has served as a model for structure-function studies of this family. Its structure was determined by X-ray crystallography as an enzyme-product complex. It consists of a novel (alphaalpha)7 barrel containing the active site that includes essential acidic residues and calcium. The structures of the subgroup 1 human endoplasmic reticulum alpha1,2-mannosidase and of a subgroup 2 fungal alpha1,2-mannosidase were determined by molecular replacement. Comparison of the enzyme structures is providing some insight into the reasons for their different specificities.

L9 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2003 ACS
AN 2001:616471 CAPLUS
DN 135:300359
TI Filamentous fungus Aspergillus oryzae has two types of .alpha.-1,2-mannosidases, one of which is a microsomal enzyme that removes a single mannose residue from Man9GlcNAc2
AU Yoshida, Takashi; Kato, Yoji; Asada, Yoshihiro; Nakajima, Tasuku
CS Faculty of Agriculture and Life Science, Hirosaki University, Aomori, 036-8561, Japan
SO Glycoconjugate Journal (2001), Volume Date 2000, 17(11), 745-748
CODEN: GLJOEW; ISSN: 0282-0080
PB Kluwer Academic Publishers
DT Journal
LA English
AB .alpha.-Mannosidase activities towards high-mannose oligosaccharides were examd. with a detergent-solubilized microsomal prepn. from a filamentous fungus, Aspergillus oryzae. In the enzymic reaction, the pyridylaminated substrate Man9GlcNAc2-PA was trimmed to Man8GlcNAc2-PA which lacked one .alpha.-1,2-mannose residue at the nonreducing terminus of the middle branch (Man8B isomer), and this mannooligosaccharide remained predominant through the overall reaction. Trimming was optimal at pH 7.0 in PIPES buffer in the presence of calcium ion and kifunensine was inhibitory with IC50 below 0.1 .mu.M. These results suggest that the activity is the same type as was previously obsd. with human and yeast endoplasmic reticulum (ER) .alpha.-mannosidases. Considering these results together with previous data on a fungal .alpha.- ***1*** , ***2*** - ***mannosidase*** that trimmed Man9GlcNAc2 to ***Man5GlcNAc2*** , the filamentous fungi appear to have two types of .alpha.-1,2-mannosidases, each of which acts differently on N-linked mannooligosaccharides.
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 7 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 2000:451973 BIOSIS

DN PREV200000451973
TI Mutation of Arg273 to Leu alters the specificity of the yeast N-glycan processing class I alpha1,2-mannosidase.
AU Romero, Pedro A.; Vallee, Francois; Howell, P. Lynne; Herscovics, Annette (1)
CS (1) McGill Cancer Centre, McGill University, 3655 Promenade Sir-William-Osler, Montreal, PQ, H3G 1Y6 Canada
SO Journal of Biological Chemistry, (April 14, 2000) Vol. 275, No. 15, pp. 11071-11074. print.
ISSN: 0021-9258.
DT Article
LA English
SL English
AB Class I alpha1,2-mannosidases (glycosyl hydrolase family 47) involved in the processing of N-glycans during glycoprotein maturation have different specificities. Enzymes in the endoplasmic reticulum of yeast and mammalian cells remove a single mannose from Man9GlcNAc2 to form Man8GlcNAc2 isomer B (lacking the alpha1, 2-mannose residue of the middle alpha1, 3-arm), whereas other alpha1,2-mannosidases, including Golgi alpha1,2-mannosidases IA and IB, can convert Man9GlcNAc2 to ***Man5GlcNAc2*** . In the present work, it is demonstrated that with a single mutation in its catalytic domain (Arg273 fwdarw Leu) the yeast endoplasmic reticulum alpha1,2-mannosidase acquires the ability to transform Man9GlcNAc to Man5GlcNAc. High resolution proton nuclear magnetic resonance analysis of the products shows that the order of removal of mannose from Man9GlcNAc is different from that of other alpha1,2-mannosidases that remove four mannose from Man9GlcNAc. These results demonstrate that Arg273 is in part responsible for the specificity of the endoplasmic reticulum alpha1,2-mannosidase and that small differences in non-conserved amino acids interacting with the oligosaccharide substrate in the active site of class I alpha1,2-mannosidases are responsible for the different specificities of these enzymes.

L9 ANSWER 8 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2000:153863 BIOSIS
DN PREV200000153863
TI Reciprocal relationship between alpha1,2 mannosidase processing and reglucosylation in the rough endoplasmic reticulum of Man-P-Dol deficient cells.
AU Duvet, Sandrine; Chirat, Frederic; Mir, Anne-Marie; Verbert, Andre; Dubuisson, Jean; Cacan, Rene (1)
CS (1) Laboratoire de Chimie Biologique, CNRS-UMR 8576, Universite des Sciences et Technologies de Lille, 59655, Villeneuve d'Ascq Cedex France
SO European Journal of Biochemistry., (Feb., 2000) Vol. 267, No. 4, pp. 1146-1152.
ISSN: 0014-2956.
DT Article
LA English
SL English
AB The study of the glycosylation pathway of a mannosylphosphoryldolichol-deficient CHO mutant cell line (B3F7) reveals that truncated Glc(0-3) ***Man5GlcNAc2*** oligosaccharides are transferred onto nascent proteins. Pulse-chase experiments indicate that these newly synthesized glycoproteins are retained in intracellular compartments and converted to Man4GlcNAc2 species. In this paper, we demonstrate that the alpha1,2 mannosidase, which is involved in the processing of ***Man5GlcNAc2***

into Man4GlcNAc2, is located in the rough endoplasmic reticulum. The enzyme was shown to be inhibited by kifunensine and deoxymannojirimycin, indicating that it is a class I mannosidase. In addition, Man4GlcNAc2 species were produced at the expense of Glc1Man5GlcNAc2 species. Thus, the trimming of ***Man5GlcNAc2*** to Man4GlcNAc2, which is catalyzed by this mannosidase, could be involved in the control of the glucose-dependent folding pathway.

L9 ANSWER 9 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:427600 BIOSIS
DN PREV200100427600
TI Filamentous fungus Aspergillus oryzae has two types of alpha-1,2-mannosidases, one of which is a microsomal enzyme that removes a single mannose residue from Man9GlcNAc2.
AU Yoshida, Takashi (1); Kato, Yoji; Asada, Yoshihiro; Nakajima, Tasuku
CS (1) Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo, Hirosaki, Aomori, 036-8561: ytakashi@cc.hirosaki-u.ac.jp Japan
SO Glycoconjugate Journal, (November, 2000) Vol. 17, No. 11, pp. 745-748. print.
ISSN: 0282-0080.
DT Article
LA English
SL English
AB alpha-Mannosidase activities towards high-mannose oligosaccharides were examined with a detergent-solubilized microsomal preparation from a filamentous fungus, Aspergillus oryzae. In the enzymatic reaction, the pyridylaminated substrate Man9GlcNAc2-PA was trimmed to Man8GlcNAc2-PA which lacked one alpha-1,2-mannose residue at the nonreducing terminus of the middle branch (Man8B isomer), and this mannooligosaccharide remained predominant through the overall reaction. Trimming was optimal at pH 7.0 in PIPES buffer in the presence of calcium ion and kifunensine was inhibitory with IC50 below 0.1 muM. These results suggest that the activity is the same type as was previously observed with human and yeast endoplasmic reticulum (ER) alpha-mannosidases. Considering these results together with previous data on a fungal alpha- ***1*** , ***2*** - ***mannosidase*** that trimmed Man9GlcNAc2 to ***Man5GlcNAc2*** (Ichishima, E., et al. (1999) Biochem J, 339: 589-597), the filamentous fungi appear to have two types of alpha-1,2-mannosidases, each of which acts differently on N-linked mannooligosaccharides.

L9 ANSWER 10 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 2000:265973 BIOSIS
DN PREV200000265973
TI N-glycan processing by a lepidopteran insect alpha1,2-mannosidase.
AU Kawar, Ziad; Romero, Pedro A.; Herscovics, Annette; Jarvis, Donald L. (1)
CS (1) Department of Molecular Biology, University of Wyoming, Laramie, WY, 82071-3944 USA
SO Glycobiology, (April, 2000) Vol. 10, No. 4, pp. 347-355. print..
ISSN: 0959-6658.
DT Article
LA English
SL English
AB Protein glycosylation pathways are relatively poorly characterized in insect cells. As part of an overall effort to address this problem, we previously isolated a cDNA from Sf9 cells that encodes an insect alpha1,2-mannosidase (SfManI) which requires calcium and is inhibited by

1-deoxymannojirimycin. In the present study, we have characterized the substrate specificity of SfManI. A recombinant baculovirus was used to express a GST-tagged secreted form of SfManI which was purified from the medium using an immobilized glutathione column. The purified SfManI was then incubated with oligosaccharide substrates and the resulting products were analyzed by HPLC. These analyses showed that SfManI rapidly converts Man9GlcNAc2 to Man6Glc-NAc2 isomer C, then more slowly converts Man6GlcNAc2 isomer C to ***Man5GlcNAc2*** . The slow step in the processing of Man9GlcNAc2 to ***Man5GlcNAc2*** by SfManI is removal of the alpha1,2-linked mannose on the middle arm of Man9GlcNAc2. In this respect, SfManI is similar to mammalian alpha1,2-mannosidases IA and IB. However, additional HPLC and 1H-NMR analyses demonstrated that SfManI converts Man9GlcNAc2 to ***Man5GlcNAc2*** primarily through Man7GlcNAc2 isomer C, the archetypal Man9GlcNAc2 missing the lower arm alpha1,2-linked mannose residues. In this respect, SfManI differs from mammalian alpha1,2-mannosidases IA and IB, and is the first alpha1,2-mannosidase directly shown to produce Man7GlcNAc2 isomer C as a major processing intermediate.

L9 ANSWER 11 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6

AN 1999:423244 BIOSIS

DN PREV199900423244

TI Identification, expression, and characterization of a cDNA encoding human endoplasmic reticulum mannosidase I, the enzyme that catalyzes the first mannose trimming step in mammalian Asn-linked oligosaccharide biosynthesis.

AU Gonzalez, Daniel S.; Karaveg, Khanita; Vandersall-Nairn, Alison S.; Lal, Anita; Moremen, Kelley W. (1)

CS (1) Dept. of Biochemistry and Molecular Biology, University of Georgia, Life Sciences Bldg., Athens, GA, 30602 USA

SO Journal of Biological Chemistry, (July 23, 1999) Vol. 274, No. 30, pp. 21375-21386.
ISSN: 0021-9258.

DT Article

LA English

SL English

AB We have isolated a full-length cDNA clone encoding a human alpha1,2-mannosidase that catalyzes the first mannose trimming step in the processing of mammalian Asn-linked oligosaccharides. This enzyme has been proposed to regulate the timing of quality control glycoprotein degradation in the endoplasmic reticulum (ER) of eukaryotic cells. Human expressed sequence tag clones were identified by sequence similarity to mammalian and yeast oligosaccharide-processing mannosidases, and the full-length coding region of the putative mannosidase homolog was isolated by a combination of 5'-rapid amplification of cDNA ends and direct polymerase chain reaction from human placental cDNA. The open reading frame predicted a 663-amino acid type II transmembrane polypeptide with a short cytoplasmic tail (47 amino acids), a single transmembrane domain (22 amino acids), and a large COOH-terminal catalytic domain (594 amino acids). Northern blots detected a transcript of apprx2.8 kilobase pairs that was ubiquitously expressed in human tissues. Expression of an epitope-tagged full-length form of the human mannosidase homolog in normal rat kidney cells resulted in an ER pattern of localization. When a recombinant protein, consisting of protein A fused to the COOH-terminal luminal domain of the human mannosidase homolog, was expressed in COS cells, the fusion protein was found to cleave only a single

alpha1,2-mannose residue from Man9GlcNAc2 to produce a unique Man8GlcNAc2 isomer (Man8B). The mannose cleavage reaction required divalent cations as indicated by inhibition with EDTA or EGTA and reversal of the inhibition by the addition of Ca2+. The enzyme was also sensitive to inhibition by deoxymannojirimycin and kifunensine, but not swainsonine. The results on the localization, substrate specificity, and inhibitor profiles indicate that the cDNA reported here encodes an enzyme previously designated ER mannosidase I. Enzyme reactions using a combination of human ER mannosidase I and recombinantGolgi mannosidase IA indicated that that these two enzymes are complementary in their cleavage of Man9GlcNAc2 oligosaccharides to ***Man5GlcNAc2*** .

L9 ANSWER 12 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1998:490652 BIOSIS
DN PREV199800490652
TI Production of human compatible high mannose-type (***Man5GlcNAc2***) sugar chains in Saccharomyces cerevisiae.
AU Chiba, Yasunori; Suzuki, Misa; Yoshida, Satoshi; Yoshida, Aruto; Ikenaga, Hiroshi; Takeuchi, Makoto (1); Jigami, Yoshifumi; Ichishima, Eiji
CS (1) Central Laboratories Key Technol., KIRIN Brewery Co. Ltd., 1-13-5 Fukuura, Kanazawa-ku, Yokohama 236-0004 Japan
SO Journal of Biological Chemistry, (Oct. 9, 1998) Vol. 273, No. 41, pp. 26298-26304.
ISSN: 0021-9258.
DT Article
LA English
AB A yeast mutant capable of producing ***Man5GlcNAc2*** human compatible sugar chains on glycoproteins was constructed. An expression vector for alpha- ***1*** , ***2*** - ***mannosidase*** with the "HDEL" endoplasmic reticulum retention/ retrieval tag was designed and expressed in Saccharomyces cerevisiae. An in vitro alpha- ***1*** , ***2*** - ***mannosidase*** assay and Western blot analysis showed that it was successfully localized in the endoplasmic reticulum. A triple mutant yeast lacking three glycosyltransferase activities was then transformed with an alpha- ***1*** , ***2*** - ***mannosidase*** expression vector. The oligosaccharide structures of carboxypeptidase Y as well as cell surface glycoproteins were analyzed, and the recombinant yeast was shown to produce a series of high mannose-type sugar chains including ***Man5GlcNAc2*** . This is the first report of a recombinant S. cerevisiae able to produce ***Man5GlcNAC2*** -oligosaccharides, the intermediate for hybrid-type and complex-type sugar chains.

L9 ANSWER 13 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
AN 1998:494817 BIOSIS
DN PREV199800494817
TI Substrate specificities of recombinant murine Golgi alpha1,2-mannosidases IA and IB and comparison with endoplasmic reticulum and Golgi processing alpha1,2-mannosidases.
AU Lal, Anita; Pang, Peng; Kalelkar, Sandeep; Romero, Pedro A.; Herscovics, Annette; Moremen, Kelley W. (1)
CS (1) Complex Carbohydrate Res. Cent., Univ. Georgia, Athens, GA 30602 USA
SO Glycobiology, (Oct., 1998) Vol. 8, No. 10, pp. 981-995.
ISSN: 0959-6658.
DT Article
LA English

AB The catalytic domains of murine Golgi alpha1,2-mannosidases IA and IB that are involved in N-glycan processing were expressed as secreted proteins in P. pastoris. Recombinant mannosidases IA and EB both required divalent cations for activity, were inhibited by deoxymannojirimycin and kifunensine, and exhibited similar catalytic constants using Manalpha1,2Manalpha-O-CH3 as substrate. Mannosidase IA was purified as a 50 kDa catalytically active soluble fragment and shown to be an inverting glycosidase. Recombinant mannosidases IA and EB were used to cleave Man9GlcNAc and the isomers produced were identified by high performance liquid chromatography and proton-nuclear magnetic resonance spectroscopy. Man9GlcNAc was rapidly cleaved by both enzymes to Man6GlcNAc, followed by a much slower conversion to Man5GlcNAc. The same isomers of Man7GlcNAc and Man6GlcNAc were produced by both enzymes but different isomers of Man8GlcNAc were formed. When Man8GlcNAc (Man8B isomer) was used as substrate, rapid conversion to Man5GlcNAc was observed, and the same oligosaccharide isomer intermediates were formed by both enzymes. These results combined with proton-nuclear magnetic resonance spectroscopy data demonstrate that it is the terminal al,2-mannose residue missing in the Man8B isomer that is cleaved from Man9GlcNAc at a much slower rate. When rat liver endoplasmic reticulum membrane extracts were incubated with Man9GlcNAc2, Man8GlcNAc2 was the major product and Man8B was the major isomer. In contrast, rat liver Golgi membranes rapidly cleaved Man9GlcNAc2 to Man6GlcNAc2 and more slowly to ***Man5GlcNAc2*** . In this case all three isomers of Man8GlcNAc2 were formed as intermediates, but a distinctive isomer, Man8A, was predominant. Antiserum to recombinant mannosidase IA immunoprecipitated an enzyme from Golgi extracts with the same specificity as recombinant mannosidase IA. These immunodepleted membranes were enriched in a Man9GlcNAc2 to Man8GlcNAc2-cleaving activity forming predominantly the Man8B isomer. These results suggest that mannosidases IA and IB in Golgi membranes prefer the Man8B isomer generated by a complementary mannosidase that removes a single mannose from Man9GlcNAc2.

L9 ANSWER 14 OF 24 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 9
AN 95074871 EMBASE
DN 1995074871
TI Purification and properties of a Golgi-derived (.alpha. ***1*** , ***2***)- ***mannosidase*** -I from baculovirus-infected lepidopteran insect cells (IPLB-SF21AE) with preferential activity toward mannose6-N-acetylglucosamine2.
AU Ren J.; Bretthauer R.K.; Castellino F.J.
CS Dept. of Chemistry/Biochemistry, University of Notre Dame,Notre Dame, IN 46556, United States
SO Biochemistry, (1995) 34/8 (2489-2495).
ISSN: 0006-2960 CODEN: BICHAW
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB Because the availability and subcellular distribution of processing mannosidases in cells play such powerful roles in determining ultimate structures of glycoconjugates, we desired to identify, characterize, and investigate possible regulation of mannosidases in infected and noninfected lepidopteran insect cells. Since our previous observations that a mannosidase activity that converted Man6GlcNAc2 to ***Man5GlcNAc2*** was enhanced in virus- infected cells, thus providing

the necessary intermediate for further processing to complex-type oligosaccharides, we attempted purification of this enzyme. A mannosidase was isolated and purified from membranes, operationally defined as Golgi, of recombinant baculovirus-infected Spodoptera frugiperda (IPLB-SF-21AE) cells. The molecular mass of this protein was approximately 63 kDa. Assays performed by measuring the conversion of NaB3H4-reduced Man6GlcNAc2-ol to Man5GlcNAc-[3H]GlcNAc2- ol demonstrated that the mannosidase activity was dependent on the presence of divalent cations, which was optimal for Ca2+ at pH 6.0. Inclusion of 1- deoxymannojirimycin resulted in 50% inhibition at a concentration of 20 .mu.M, whereas swainsonine did not show such inhibition. No activity was observed with p-nitrophenyl .alpha.-D-mannoside (4 mM) as a substrate. The preferred reduced oligosaccharide substrate was Man6GlcNAc2-ol, with lower activities obtained with Man9GlcNAc2-ol, Man8GlcNAc2-ol, and Man7GlcNAc2-ol. With Man6GlcNAc2-ol as substrate, products smaller than reduced ***Man5GlcNAc2*** - ol were not observed. Mannose was also liberated from the glycoprotein, ovalbumin. These properties are consistent with an enzyme classification as a type I (.alpha.1,2)-Man6-mannosidase.

L9 ANSWER 15 OF 24 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 10
AN 95346469 EMBASE
DN 1995346469
TI Mannosyltransferase activities in membranes from various yeast strains.
AU Verostek M.F.; Trimble R.B.
CS Wadsworth Center C-535, New York State Department of Health, PO Box 509,Albany, NY 12201-0509, United States
SO Glycobiology, (1995) 5/7 (671-681).
ISSN: 0959-6658 CODEN: GLYCE3
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB In the yeast Golgi compartments, at least five, and potentially several additional mannosyltransferases are involved in elongating to 'mannan' the core Man8GlcNAc2 oligosaccharide trimmed from Glc3Man9GlcNAc2 in the endoplasmic reticulum. Structural studies on oligosaccharides from alg3 mutant yeast, which lack the four upper arm mannoses donated by Man-P-Dol (where Dol is dolichol), verified that the new .alpha.1,6-branch in endo H-resistant mannan in this strain is efficiently initiated in vivo on the .alpha.1,3-linked core residue of the lipid-oligosaccharide form of ***Man5GlcNAc2*** (Verostek et al., J. Biol, Chem., 266, 5547-5551, 1991). This Man5GlcNAcGlcNAc[3H]ol isomer (where GlcNAc[3H]ol is N-acetylglucosamin [1-3H] itol) was found to be an excellent acceptor for a number of GDP-Man-dependent Golgi mannosyltransferases in detergent-solubilized yeast membrane preparations: an .alpha.1,3-mannosyltransferase (Mnn1p), an .alpha.1,6-mannosyltransferase (Och1p) and two .alpha.1,2mannosyltransferases (Mnt1p/Kre2p, ?) whose products were readily identified by 1H NMR spectroscopy. The Man6GlcNAcGlcNAc[3H]ol isomers formed were easily defined by .alpha. ***1*** , ***2*** - ***mannosidase*** sensitivity and either Bio-Gel P-4 gel filtration or AX-5 high-performance liquid chromatography. In general, mannosyltransferases present in detergent-solubilized microsomes from most yeast strains mimicked the array of sugar linkages observed on their respective glycoproteins. However, in the case of the Saccharomyces pmr1 mutant, an .alpha.1,3-mannosyltransferase was active in microsomal

extracts, but the .alpha.1,3-Man epitope could not be identified on Western blots of cellular glycoproteins using sugar linkage-specific antibodies or lectins. The in vitro transferase assay is simple, rapid and accurate, and in the case of pmr1 suggests that in vivo either invertase is misrouted during secretion or the .alpha.1,3-mannosyltransferase is mistargeted after its synthesis in this mutant.

L9 ANSWER 16 OF 24 CAPLUS COPYRIGHT 2003 ACS
AN 1994:185868 CAPLUS
DN 120:185868
TI A 1,2-.alpha.-D-mannosidase from a Bacillus sp.: purification, characterization, and mode of action
AU Maruyama, Yutaka; Nakajima, Tasuku; Ichishima, Eiji
CS Fac. Agric., Tohoku Univ., Sendai, 981, Japan
SO Carbohydrate Research (1994), 251, 89-98
CODEN: CRBRAT; ISSN: 0008-6215
DT Journal
LA English
AB 1,2-.alpha.-Mannosidase (I) was purified to homogeneity from the culture supernatant of Bacillus sp. M-90, which was isolated from soil by enrichment culture on bakers' yeast mannan. Purified I had a mol. wt. of 380 kDa, and was comprised of 2 apparently identical 190-kDa subunits. I had a neutral optimum pH (7.0) and a pI of 3.6. I was highly specific for .alpha.-1,2-linked D-mannose oligosaccharides. An N-linked high-mannose type oligosaccharide, Man9GlcNAc2, was a good substrate, yielding ***Man5GlcNAc2*** , and the .alpha.-1,2-linked side-chains of Saccharomyces cerevisiae mannan were also specifically hydrolyzed by the enzyme. P-nitrophenyl .alpha.-D-mannopyranoside and 1,2-.alpha.-D-mannobiitol were not hydrolyzed by I. Ca2+, 1-deoxymannojirimycin, and swainsonine had no effect on I, but I was completely inhibited by EDTA. The mode of action on .alpha.-1,2-linked mannotetraose indicated that I was an exoenzyme.

L9 ANSWER 17 OF 24 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 11
AN 93168190 EMBASE
DN 1993168190
TI Glycoprotein biosynthesis in the alg3 Saccharomyces cerevisiae mutant. I. Role of glucose in the initial glycosylation of invertase in the endoplasmic reticulum.
AU Verostek M.F.; Atkinson P.H.; Trimble R.B.
CS Wadsworth Center for Labs/Research, New York State Department of Health, P. O. Box 509,Albany, NY 12201-0509, United States
SO Journal of Biological Chemistry, (1993) 268/16 (12095-12103).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB Oligosaccharides on invertase restricted to the endoplasmic reticulum (ER) in alg3,sec18 yeast at 37 .degree.C were found to be 20% wild type Man8GlcNAc and 80% Man1.alpha..fwdarw.2Man1.alpha..fwdarw.2Man1.alpha..fwdarw.3(Man1.alpha..fwdarw.6)Man1.beta..fwdarw.4GlcNAc2 (Verostek, M. F., Atkinson, P. H., and Trimble, R. B. (1991) J. Biol. Chem. 266, 5547-5551). These results suggested that alg3 was slightly leaky, but did not address whether the oligosaccharide-lipid Man9GlcNAc2 and ***Man5GlcNAc2*** precursors were glucosylated in alg3 yeast. Therefore, an alg3,sec18,gls1

strain was constructed to delete the GLS1-encoded glucosidase I responsible for trimming the terminal .alpha.1,2-linked glucose from newly transferred Glc3Man(x)GlcNAc2 oligosaccharides. Invertase activity was overexpressed 5-10-fold on transforming this strain with a multicopy plasmid (pRB58) carrying the SUC2 gene, and preparative amounts of the ER form of external invertase, derepressed and accumulated at 37 .degree.C, were purified. The N-linked glycans were released by sequential treatment with endo-.beta.-N-acetyl-glucosaminidase H (endo H) and peptide-N4-N-acetyl-.beta.-glucosaminyl asparagine amidase. Oligosaccharide pools were sized separately on Bio-Gel P-4, which showed that endo H released about 17% of the carbohydrate as Glc3Man8GlcNAc, while peptide-N4-N-acetyl-.beta.-glucosaminyl asparagine amidase released the remainder as Hex8GlcNAc2 and ***Man5GlcNAc2*** in a 1:4 ratio. Glycan structures were assigned by 500-MHz two-dimensional DQF-COSY 1H NMR spectroscopy, which revealed that the endo H-resistant Hex8GlcNAc2 pool contained Glc3Man5GlcNAc2 and Man8GlcNAc2 in a 6:4 ratio, the latter a different isomer from that formed by the ER .alpha. ***1*** , ***2*** - ***mannosidase*** (Byrd, J. C., Tarentino, A. L., Maley, F., Atkinson, P. H., and Trimble, R. B. (1982) J. Biol. Chem. 257, 14657-14666). Recovery of Glc3Man8GlcNAc and not the ER form of Man8GlcNAc provided an internal control indicating the absence of glucosidase I, which was confirmed by incubation of [3H]Glc3[14C]Man9GlcNAc with solubilized membranes from either alg3,sec18,gls1 or alg3,sec18,GLS1 strains. Chromatographic analysis of the products showed that [3H]Glc was removed only in the presence of the GLS1 gene product. Thus, the vast majority of the N-linked glycosylation in the ER of alg3 yeast (>75%) occurs by transfer of ***Man5GlcNAc2*** without prior addition of the 3 glucoses normally found on the lipid-linked precursor.

L9 ANSWER 18 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
AN 1991:227378 BIOSIS
DN BA91:118838
TI STRUCTURE OF SACCHAROMYCES-CEREVISIAE ALG3 SEC 18 MUTANT OLIGOSACCHARIDES.
AU VEROSTEK M F; ATKINSON P H; TRIMBLE R B
CS WADSWORTH CENT. LAB. RES., NEW YORK STATE DEP. HEALTH, P.O. BOX 509, ALBANY, N.Y. 12201-0509.
SO J BIOL CHEM, (1991) 266 (9), 5547-5551.
CODEN: JBCHA3. ISSN: 0021-9258.
FS BA; OLD
LA English
AB Asparagine-linked oligosaccharides are synthesized by transfer of Glc3Man9GlcNAc2 from dolichol pyrophosphate proceeds by highly ordered sequential addition of mannose and glucose to form Glc3Man9GlcNAc2-P-P-dolichol. Yeast mutants in asparagine-linked glycosylation (alg), generated by an 3H-Man suicide technique, were assigned to eight complementation groups which define steps in oligosaccharide-lipid synthesis (Huffaker, T.C., and Robbins, P.W. (1982) J. Biol. Chem. 257, 3203-3210). Alg3 invertase oligosaccharides are resistant to endo-.beta.-N-acetylglucosaminidase H, and the lipid-oligosaccharide pool yields Man5Glc-NAc2, suggesting its structure may be that from mammalian cells lacking Man-P-dolichol (Chapman, A., et al. (1980) J. Biol. Chem. 255, 4441-4446). To test this supposition the endoplasmic reticulum form of invertase derepressed in alg3,sec 18 yeast at 37.degree. C was isolated as a source of oligosaccharides whose processing beyond glucose and/or mannose trimming, if involved, would be prevented. Man8GlcNAc2 and ***Man5GlcNAc2*** were released by peptide-N-glycosidase F from

alg3,sec18 invertase in a 1:5 molar ratio. 1HNMR spectroscopy revealed Man8GlcNAc2 to be the .alpha. ***1*** , ***2*** - ***mannosidase*** -trimming product described earlier (Byrd, J.C., Tarentino, A.L., Maley, F., Atkinson, P.H., and Trimble, R.B. (1982) J. Biol. Chem. 257, 14657-14666), while ***Man5GlcNAc2*** was Man.alpha.1,2Man.alpha.1,2Man.alpha.1,3(Man.alpha.1,6)Man.beta.1, 4GlcNAc.beta.1,4GlcN Ac. This provides a structural proof for the lipid-linked ***Man5GlcNAc2*** originally proposed from enzymatic and chemical analyses of the radiolabeled mammalian precursor. Experimental evidence indicates that, unlike the mammalian cell mutants which are unable to synthesize Man-P-dolichol, alg3 yeast accumulate ***Man5GlcNAc2*** -P-P-dolichol due to a defective .alpha.1,3-mannosyltransferase required for the next step in oligosaccharide-lipid elongation.

L9 ANSWER 19 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 13
AN 1991:502940 BIOSIS
DN BA92:125900
TI IN-VITRO HYDROLYSIS OF OLIGOMANNOSE-TYPE SUGAR CHAINS BY AN ALPHA-***1*** ***2*** ***MANNOSIDASE*** FROM MICROSOMES OF DEVELOPING CASTOR BEAN COTYLEDONS.
AU KIMURA Y; YAMAGUCHI O; SUEHISA H; TAKAGI S
CS DEP. AGRIC. SCI., FAC. AGRIC., OKAYAMA UNIV., TSUSHIMA-NAKA, OKAYAMA 700, JPN.
SO BIOCHIM BIOPHYS ACTA, (1991) 1075 (1), 6-11.
CODEN: BBACAQ. ISSN: 0006-3002.
FS BA; OLD
LA English
AB An .alpha.- ***1*** , ***2*** - ***mannosidase*** involved in the processing of N-linked oligosaccharides was prepared from the microsomal fraction of developing castor bean cotyledons. The processing .alpha.-mannosidase was solubilized with 1.0% Triton X-100 and purified by ion-exchange chromatography followed by two gel filtration steps. The enzyme obtained could convert Man9GlcNAc2-PA to ***Man5GlcNAc2*** -PA, but this enzyme was inactive with ***Man5GlcNAc2*** -PA, Man4GlcNAc2-PA, and p-nitrophenyl-.alpha.-D-mannopyranoside. The enzyme was optimally active between pH 5.5-6.0. The processing mannosidase was inhibited by deoxymannojirimycin, EDTA, and Tris ions but not by swainsonine. Structural analyses of the mannose-trimming intermediates produced by the .alpha.-mannosidase revealed that specific intermediates were formed during conversion of Man9GlcNAc2-PA to ***Man5GlcNAc2*** -PA.

L9 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2003 ACS
AN 1989:190108 CAPLUS
DN 110:190108
TI Brefeldin A inhibits oligosaccharide processing of glycoproteins in mouse hypothyroid pituitary tissue at several subcellular sites
AU Perkel, Victor S.; Miura, Yoshitaka; Magner, James A.
CS Michael Reese Hosp., Univ. Chicago, Chicago, IL, 60616, USA
SO Proceedings of the Society for Experimental Biology and Medicine (1989), 190(3), 286-93
CODEN: PSEBAA; ISSN: 0037-9727
DT Journal
LA English
AB The effects of brefeldin A (BFA) and monensin on the processing of the

oligosaccharides of TSH, free .alpha.-subunits , and cellular glycoproteins of mouse pituitary tissue were studied to clarify the subcellular sites of action of BFA. Pituitaries from hypothyroid mice were incubated with [35S]methionine, [3H]mannose, [3H]galactose, [3H]fucose, N-[3H]acetylmannosamine, or [35S]sulfate for 2 h in the absence or presence of 5 .mu.g of BFA/mL or 2 .mu.M monensin. TSH and free .alpha.-subunits were immunopptd. from tissue lysates and analyzed by SDS-PAGE. The tryptic glycopeptides of TSH were sepd. by using HPLC. Total glycoproteins in cell lysates were pptd. with TCA. Labeled oligosaccharides were released from the tryptic glycopeptides of TSH and cellular glycoproteins by endoglycosidase H, and they were analyzed by paper chromatog. Compared with control incubations, BFA caused the intracellular accumulation of glycoproteins having less than expected amts. of Man9GlcNAc2 units, but with excess Man8GlcNAc2, Man7GlcNAc2, Man6GlcNAc2, and ***Man5GlcNAc2*** units. There was a lesser accumulation of glucose-contg. oligosaccharides, esp. Glc1Man9GlcNAc2. Monensin also caused the accumulation of certain high-mannose species, but the pattern differed from that seen for BFA, since Man9GlcNAc2 units were preserved and there was less excess of Man8GlcNAc2, Man7GlcNAc2, Man6GlcNAc2, and ***Man5GlcNAc2*** units. BFA did not block the initial attachment of oligosaccharides at any of the 3 Asn-glycosylation sites of TSH, but caused the accumulation of Man5-8GlcNAc2 units at each site. Both monensin and BFA inhibited fucosylation, sulfation, and sialylation more markedly than mannose incorporation. Thus, in addn. to its previously described action of inhibiting rough endoplasmic reticulum-to-Golgi transport, BFA apparently partially inhibits the glucose-trimming enzymes as well as some Golgi enzymes.

L9 ANSWER 21 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 14
AN 1988:374054 BIOSIS
DN BA86:57964
TI THE EFFECTS OF BREFELDIN-A ON THE HIGH MANNOSE OLIGOSACCHARIDES OF MOUSE TSH FREE ALPHA-SUBUNITS AND TOTAL GLYCOPROTEINS.
AU PERKEL V S; LIU A Y; MIURA Y; MAGNER J A
CS DIV. ENDOCRINOL., MICHAEL REESE HOSP., LAKE SHORE DRIVE AT 31ST ST., CHICAGO, ILLINOIS 60616.
SO ENDOCRINOLOGY, (1988) 123 (1), 310-318.
CODEN: ENDOAO. ISSN: 0013-7227.
FS BA; OLD
LA English
AB We have studied the effects of Brefeldin-A (BFA) on the processing of high mannose (Man) oligosaccharides of TSH. BFA is a drug that inhibits the intracellular translocation of newly synthesized glycoproteins and causes dilatation of the rough endoplasmic reticulum (RER) as well as mild swelling of the Golgi apparatus. Mouse pituitary thyrotropic tumor tissue was incubated with [3H]Man for a 2-h pulse, with and without a 3-h chase; BFA (5 .mu.g/ml) was included during selected pulse and selected chase incubations. TSH and free .alpha.-subunits were obtained from detergent lysates of tissue by immunoprecipitation using specific antisera. Total glycoproteins were obtained by trichloroacetic acid precipitation. Endoglycosidase-H-released [3H]oligosaccharides were analyzed by paper chromatography. BFA inhibited carbohydrate processing of TSH, free .alpha.-subunits, and total glycoproteins, resulting in the accumulation of Man8GlcNAc2, Man7GlcNAc2, Man6GlcNAc2, and ***Man5GlcNAc2*** , especially during the chase period. Subcellular fractions enriched in RER, heavy (proximal) Golgi, and light (distal)Golgi were prepared by

centrifugation in discontinuous sucrose gradients. [3H]Man-labeled oligosaccharides of TSH and total glycoproteins in the subcellular fractions were analyzed. In contrast to oligosaccharides with eight or nine Man residues found in control incubations, BFA caused the accumulation of oligosaccharides containing five to eight Man residues. These BFA-induced oligosaccharide alterations began in the RER and proximal Golgi with the 2-h pulse and extended into the distal Golgi during the chase incubations. Thus BFA blocks the normal intracellular transport and processing of TSH, free .alpha.-subunits, and total glycoproteins within thyrotrophs, causing species with smaller than normal high Man oligosaccharides to appear in subcellular compartments as early as the RER. The translocation block between RER and Golgi produced by BFA may prevent the processing of Man8GlcNAc2 to ***Man5GlcNAc2*** by Golgi (.alpha., ***1*** - ***2***) ***mannosidase*** I, yet the species retained within the RER may be subject to ongoing processing by endoplasmic reticulum (.alpha., ***1*** - ***2***) ***mannosidase*** , resulting in the accumulation of Man5-8GlcNAc2 within the RER.

L9 ANSWER 22 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1985:345204 BIOSIS
DN BA80:15196
TI POSTTRANSLATIONAL PROTEIN MODIFICATION BIOSYNTHETIC CONTROL MECHANISMS IN THE GLYCOSYLATION OF THE MAJOR MYELIN GLYCOPROTEIN BY SCHWANN CELLS.
AU PODUSLO J F
CS MEMBRANE BIOCHEM. LAB., PERIPHERAL NERVE CENT., NEUROL. BIOCHEM., MAYO CLIN., MAYO FOUNDATION, ROCHESTER, MINN. 55905, U.S.A.
SO J NEUROCHEM, (1985) 44 (4), 1194-1206.
CODEN: JONRA9. ISSN: 0022-3042.
FS BA; OLD
LA English
AB The posttranslational processing of the asparagine-linked oligosaccharide chain of the major myelin glycoprotein (P0) by Schwann cells was evaluated in the permanently transected adult rat sciatic nerve, where there is no myelin assembly, and in the crush injured nerve, where there is myelin assembly. Pronase digestion of acrylamide gel slices containing the vitro labeled [3H]mannose and [3H]fucose P0 after electrophoresis permitted analysis of the glycopeptides by lectin affinity and gel filtration chromatography. The concanavalin A-Sepharose profile of the [3H]mannose P0 glycopeptides from the transected nerve revealed the high-mannose-type oligosaccharide as the predominant species (72.9%), whereas the normally expressed P0 glycoprotein that is assembled into the myelin membrane in the crushed nerve contains 82.9-91.9% of the [3H]mannose radioactivity as the complex-type oligosaccharide chain. Electrophoretic analysis of immune precipitates verified the [3H]mannose as being incorporated into P0 for both the transected and crushed nerve. The high-mannose-type glycopeptides of the transected and crushed nerve isolated from the concanavalin A-Sepharose column were hydrolyzed by endo-.beta.-N-acetylglucosaminidase H, and the oligosaccharides were separated on Biogel P4. Man8GlcNAc [N-acetylglucosamine] and Man-GlcNAc were the predominant species with radioactivity ratios of 12.5/7.2/1.4/1.0 for the Man8, Man7, Man6 and Man5 oligosaccharides, respectively. Jack bean .alpha.-D-mannosidase gave the expected yields of free Man and ManGlcNAc from these high-mannose-type oligosaccharides. At least two .alpha.-1,2-mannosidases are responsible for converting Man9GlcNAc2 to ***Man5GlcNAc2*** . Evidently, there are distinct roles for each mannosidase and the 2nd mannosidase (I-B) may be an important rate-limiting step in the processing of this glycoprotein with the resulting accumulation of Man8GlcNAc2 and Man7GlcNAc2

intermediates. Pulse chase experiments demonstrated further processing of this high-mannose-type oligosaccharide in the transected nerve. The [3H]mannose P0 glycoprotein with MW of 27,700 having the predominant high-mannose-type oligosaccharide shifted its MW to 28,500 with subsequent chase. This band at 28,500 had the complex-type oligosaccharide chain and contained fucose attached to the core asparagine-linked GlcNAc residue. The extent of oligosaccharide processing of this down-regulated glycoprotein remains to be determined.

L9 ANSWER 23 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15
AN 1986:126761 BIOSIS
DN BA81:37177
TI CHARACTERIZATION OF MICROSOMAL AND CYTOSOLIC ALPHA-1 2 MANNOSIDASES FROM MUNG BEAN HYPOCOTYLS.
AU FORSEE W T
CS DEP. BIOCHEM., UNIV. ALA. BIRMINGHAM, BIRMINGHAM, ALA. 35294.
SO ARCH BIOCHEM BIOPHYS, (1985) 242 (1), 48-57.
CODEN: ABBIA4. ISSN: 0003-9861.
FS BA; OLD
LA English
AB Microsomal and cytosolic .alpha.-mannoside activities, which hydrolyze .alpha.-1,2-mannosyl-mannose linkages in the ***Man5GlcNAc2*** oligosaccharide, have been isolated from homogenates of mung bean hypocotyls. The .alpha.- ***1*** , ***2*** - ***mannosidase*** activities were readily distinguished from previously described aryl .alpha.-mannosidases by several criteria. They were optimally active in the presence of Ca2+ between pH 5.5 and 6, they were inhibited by Zn2+, and they had essentially no activity with p-nitrophenyl-.alpha.-mannoside. The microsomal and cysolic .alpha.-1,2-mannosidases demonstrates specificity for oligosacchrides with terminal nonreducing .alpha.-1,2-mannosyl linkages, and they were inhibited by mannosyl-mannose disaccharides, with the inhibition decreasing in the order of .alpha.-1,2-> .alpha.-1,3- > .alpha.-1,6-mannosyl-mannose. The cystolic .alpha.- ***1*** , ***2*** - ***mannosidase*** activity, which was present in the 100,000 g supernatant, was separated from the aryl .alpha.-mannosidase by ammonium sulfate precipitation. The microsomal .alpha.-1,2-mannoside, which was tightly associated with the particulate fraction, was solubilized with Triton X-100 and 0.2 M KCl. The two .alpha.-1,3-mannosidase activities were readily differentiated by gel-filtration chromatography. The solubilized microsomal enzyme chromatographed in approximately the same position as a Mr 460,000 globular protein whereas the cytosolic enzyme was eluted in a retarded position, indicating a much smaller protein.

L9 ANSWER 24 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 16
AN 1984:274974 BIOSIS
DN BA78:11454
TI PROCESSING OF MOPC-315 IMMUNO GLOBULIN A OLIGO SACCHARIDES EVIDENCE FOR ENDOPLASMIC RETICULUM AND GOLGI TRANS-ALPHA- ***1*** ***2*** ***MANNOSIDASE*** ACTIVITY.
AU HICKMAN S; THEODORAKIS J L; GRECO J M; BROWN P H
CS DIV. HEMATOLOGY/ONCOLOGY, JEWISH HOSPITAL ST. LOUIS, WASHINGTON UNIV. SCH. MED., ST. LOUIS, MO. 63110.
SO J CELL BIOL, (1984) 98 (2), 407-416.
CODEN: JCLBA3. ISSN: 0021-9525.

FS BA; OLD
LA English
AB The processing of asparagine-linked oligosaccharides on the .alpha.-chains of an IgA was investigated using MOPC 315 murine plasmacytoma cells. These cells secrete IgA containing complex-type oligosaccharides that were not sensitive to endo-.beta.-N-acetylglucosaminidase H. Oligosaccharides present on the intracellular .alpha.-chain precursor were of the high mannose-type, remaining sensitive to endo-.beta.-N-acetylglucosaminidase H despite a long intracellular half-life of 2-3 h. The major [3H]mannose-labeled .alpha.-chain oligosaccharides identified after a 20-min pulse were Man8GlcNAc2 and Man9GlcNAc2. Following chase incubations, the major oligosaccharide accumulating intracellularly was Man6GlcNAc2, which contained a single .alpha.1,2-linked mannose residue. Conversion of Man6GlcNAc2 to complex-type oligosaccharides occurred at the time of secretion since appreciable amounts of ***Man5GlcNAc2*** or further processed structures could not be detected intracellularly. Subcellular locations of the .alpha. ***1*** , ***2*** - ***mannosidase*** activities were studied using carbonyl cyanide m-chlorophenylhydrazone and monensin. Despite inhibiting the secretion of IgA, these inhibitors of protein migration did not effect the initial processing of Man9GlcNAc2 to Man6GlcNAc2. No large accumulation of ***Man5GlcNAc2*** occurred, indicating the presence of 2 subcellular locations of .alpha. ***1*** , ***2*** - ***mannosidase*** activity involved in oligosaccharide processing in MOPC 315 cells. The first 3 .alpha.1,2-linked mannose residues were removed shortly after the .alpha.-chain was glycosylated, most likely in rough endoplasmic reticulum, since this processing occurred in the presence of carbonyl cyanide m-chlorophenylhydrazone. The removal of the final .alpha.1,2-linked mannose residue as well as subsequent carbohydrate processing occurred just before IgA secretion, most likely in the trans Golgi complex since processing of Man6GlcNAc2 to ***Man5GlcNAc2*** was greatly inhibited in the presence of monensin.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	97.74	97.95
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-3.91	-3.91

STN INTERNATIONAL LOGOFF AT 14:01:31 ON 05 JUN 2003